SCIENTIFIC LETTER

Novel anti-inflammatory effect of statins: reduction of $CD4^+CD28^{null}$ T lymphocyte frequency in patients with unstable angina

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Statins, inhibitors of 3-hydroxy-3-methyl glutaryl coenzyme A reductase, are potent inhibitors of cholesterol biosynthesis and have greatly improved the management of ischaemic heart disease. Recent studies suggest that direct antithrombotic and anti-inflammatory effects associated with treatment with statins may at least partly account for the reduction of cardiovascular events. In particular, statins reduce high sensitive C reactive protein (hs-CRP), tumour necrosis factor α, and metalloproteinase 9 production.[1] Recently it has been shown that statins can modify the balance of T helper subset 1 (Th 1) and 2 (Th 2) cells by inhibition of Th1 development and augmentation of Th2 development of $CD4^+$ T cells.[2] In the present observational retrospective study we observed a relation between statin use and frequency of an unusual Th1 subset of T lymphocytes, $CD4^+CD28^{null}$, which is often expanded in unstable angina.[3]

METHODS

Population

We studied 111 consecutive patients (mean (SD) age 64 (9) years; 32 women) admitted to our coronary care unit with Braunwald class IIIB unstable angina: 33 had been taking statins for at least four weeks plus standard anti-ischaemic treatment (group 1: mean age 62 (9) years; seven women); the remaining 78 patients were taking standard anti-ischaemic treatment but not statins before admission (group 2: mean (SD) age 65 (9) years; 25 women). Clinical features were recorded for both groups (table 1). All participants gave their informed consent. The ethics committee of the Catholic University of Rome approved the study.

Protocol

Blood samples were taken from all patients at the time of coronary care unit admission to measure $CD4^+CD28^{null}$ T cells and hs-CRP. Peripheral blood was stained with phycoerythrin conjugated anti-CD4 (Becton Dickinson, San Jose, California, USA) and fluorescein isothiocyanate conjugated anti-CD28 (Pharmigen, San Diego, California, USA) monoclonal antibodies and analysed on a Coulter flow cytometer. The frequency of $CD4^+CD28^{null}$ T cells was determined by flow cytometry and WinMDI software (Joseph Trotter, Scripps Research Institute, La Jolla, California, USA) and expressed as the percentage of total $CD4^+$ T cells. hs-CRP was measured with a latex enhanced immunonephelometric assay by a BN II analyser (Dade-Behring, Marburg, Germany), as described elsewhere.

Statistical analysis

Data are expressed as median and range. As the value of $CD4^+CD28^{null}$ T cells is usually expressed as the percentage of total CD4+ T cells, which reflects the individual value for each patient, and as $CD4^+CD28^{null}$ T cell frequency and CRP concentrations were not normally distributed, the non-parametric Mann-Whitney test was used to compare the groups and Spearman's test was used for correlation between two variables. Forward multivariable analysis was performed to identify independent predictors of $CD4^+CD28^{null}$ T cell frequency. We used $CD4^+CD28^{null}$ T cell frequency as the covariate variable and hs-CRP, age, sex, hypertension, hypercholesterolaemia, smoking, abdominal obesity, diabetes, and use of statins as dependent variables. The same dependent variables were used to identify independent predictors of hs-CRP. A two tailed $p < 0.05$ was considered significant.

RESULTS

All patients in group 1 had been taking statins for at least one month (median 45 (7) days). Previous use of statins was evaluated by interviewing patients. Twenty six patients (79%) took 20 mg/day atorvastatin, four patients (12%) took simvastatin 20 mg/day, and the remaining three patients (9%) took pravastatin 10 mg/day. No clinical differences were found between the two groups (table 1) or between patients taking different statins. The percentage of total lymphocytes was similar in the two groups (group 1: 26.5% (5.7–42.4%);

Table 1 Demographic and clinical characteristics of patients

	Group 1 (n = 33)	Group 2 (n = 78)
Age (median; range)	62 (43–78)	65 (44–79)
Sex (male/female)	78%/21%	67%/32%
Risk factors		
Hypertension	51%	43%
Hypercholesterolaemia	60%	50%
Smoking	46%	36%
Abdominal obesity	18%	17%
Diabetes	13%	16%
Medication		
β Blockers	67%	70%
Aspirin	76%	82%
Other antiplatelet agents	24%	18%
ACE inhibitors	40%	35%
Previous AMI	34%	36%
Duration of IHD before admission (months)		
<2	22%	18%
>2	78%	82%
No of vessels involved		
1	19%	17%
2	42%	37%
3	39%	46%
Total	100%	100%

All differences non-significant.
ACE, angiotensin converting enzyme; AMI, acute myocardial infarction; IHD, ischaemic heart disease.

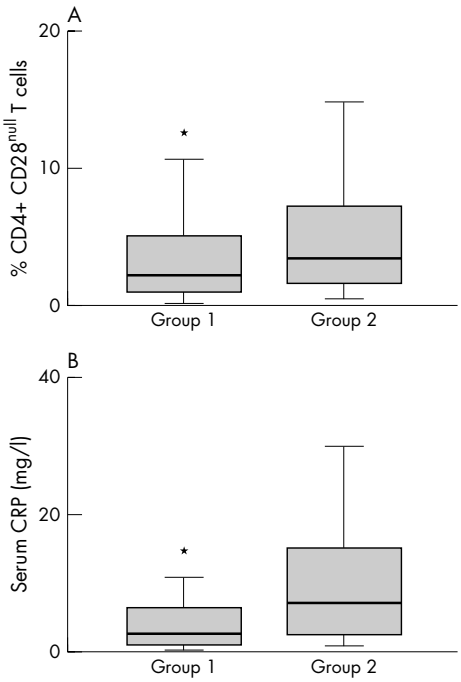

Figure 1 (A) Distribution of percentage of $CD4^+CD28^{null}$ lymphocytes in each patient, analysed by flow cytometry, was significantly lower in patients taking a statin before admission (group 1) than in patients taking standard anti-ischaemic treatment only (group 2). *p = 0.022 group 1 *v* group 2. (B) Among patients taking statins on admission (group 1) C reactive protein (CRP) concentrations, measured by high sensitivity immunonephelometric assay, were significantly lower in patients taking statins on admission than in patients not treated with statins (group 2). *p = 0.013 group 1 *v* group 2. Limits of boxes and whiskers indicate interquartile ranges, and the central lines indicate the median.

group 2: 28.7% (6.2–56.2%); not significant). The percentage of $CD4^+$ T lymphocytes also was similar in the two groups (group 1: 50.4% (12.9–70.2%); group 2: 48.8% (33.6–67.8%); not significant). The frequency of $CD4^+CD28^{null}$ T cells was significantly lower in group 1 (2.3% (0.17–21.86%)) than in group 2 (3.0% (0.56–22.9%), p = 0.022) (fig 1A). hs-CRP concentrations also were significantly lower in group 1 (4.13 mg/l (0.192–63.79 mg/l)) than in group 2 (7.17 mg/l (0.8–65.1 mg/l), p = 0.013) (fig 1B). Accordingly, at multivariable analysis independent predictors of a lower $CD4^+CD28^{null}$ T lymphocyte frequency were previous use of statins (p = 0.006) and younger age (p = 0.046). At forward multivariable analysis independent predictors of hs-CRP serum concentrations were previous use of statins (p = 0.015) and abdominal obesity (p = 0.041). No correlation was found between $CD4^+CD28^{null}$ and hs-CRP concentrations.

DISCUSSION

Our data suggest the possible correlation between statin use and reduction of the frequency of $CD4^+CD28^{null}$ T lymphocytes in patients with unstable angina and confirm an association between statin use and reduced concentrations of hs-CRP. $CD4^+CD28^{null}$ T cells, characterised by a prominent Th1 phenotype, result from long term antigenic stimulation, as they are typically oligoclonal. Candidate antigens probably derive from a microorganism chronically infecting the host or from chronic stimulation of the host's immune system by antigens such as human heat shock protein 60 or oxidised low density lipoprotein.[4] However, Zal *et al*[5] found that human heat shock protein 60, but not oxidised low density lipoprotein, is an antigen recognised by $CD28^{null}$ cells in patients with acute coronary syndromes. In patients with unstable angina increased circulating concentrations of cytokines and in particular of tumour necrosis factor α may contribute to the emergence of $CD28^{null}$ T cells by inducing loss of CD28. This T cell subset, known to be expanded in the elderly,[4] in patients with rheumatoid arthritis,[4] and in patients with unstable angina, has been found in unstable coronary plaques and may directly contribute to plaque instability through the release in a plaque microenvironment of large amounts of proinflammatory cytokines, in particular interferon γ, a potent stimulator of macrophages producing tissue destructive metalloproteinases, perforin, and granzyme B.[3] Furthermore, $CD4^+CD28^{null}$ T lymphocytes have cytolytic effects on endothelial cells, amplified by CRP.[6] Our observation of reduced concentrations of this particular population of T lymphocytes in patients admitted to the coronary care unit with unstable angina who were taking statins before admission may indicate that statins have an important immunomodulatory effect. New and prospective studies are warranted to confirm the findings of our observational retrospective study. Our observation may have clinical importance because of the $CD4^+CD28^{null}$ T cell proinflammatory and plaque damaging potential and because of the described higher recurrence of acute coronary events in patients with raised concentrations of $CD4^+CD28^{null}$ T cells. We have described one of the pleiotropic mechanisms of statins leading to reduction of short to medium term risk in patients with acute coronary syndromes, as shown in recent trials.

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